4,6-alpha-Glucanotransferase activity occurs more widespread in Lactobacillus strains and constitutes a separate GH70 subfamily

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Supplemental data

Structural characterization of linear isomalto/malto-oligomer products synthesized by the novel GTFB 4,6-α-glucanotransferase enzyme from *Lactobacillus reuteri* 121

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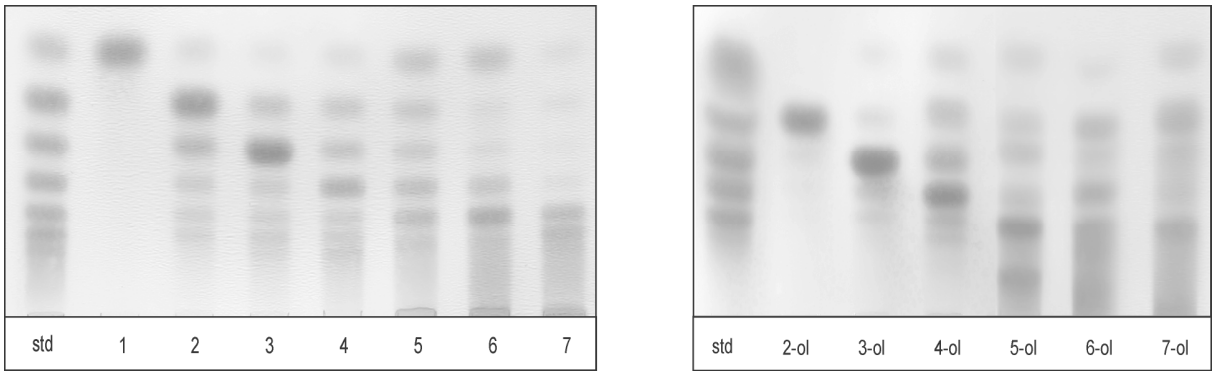

Fig. S1. TLC showing the GTFB activity on different malto-oligosaccharides(-alditols). [DP1, DP2(-ol), DP3(-ol), DP4(-ol), DP5(-ol), DP6(-ol), DP7(-ol)]. Incubations of 100 mM oligosaccharide(-alditol) solutions were carried out with 500 nM GTFB for 72 h at 37°C and pH 4.7.

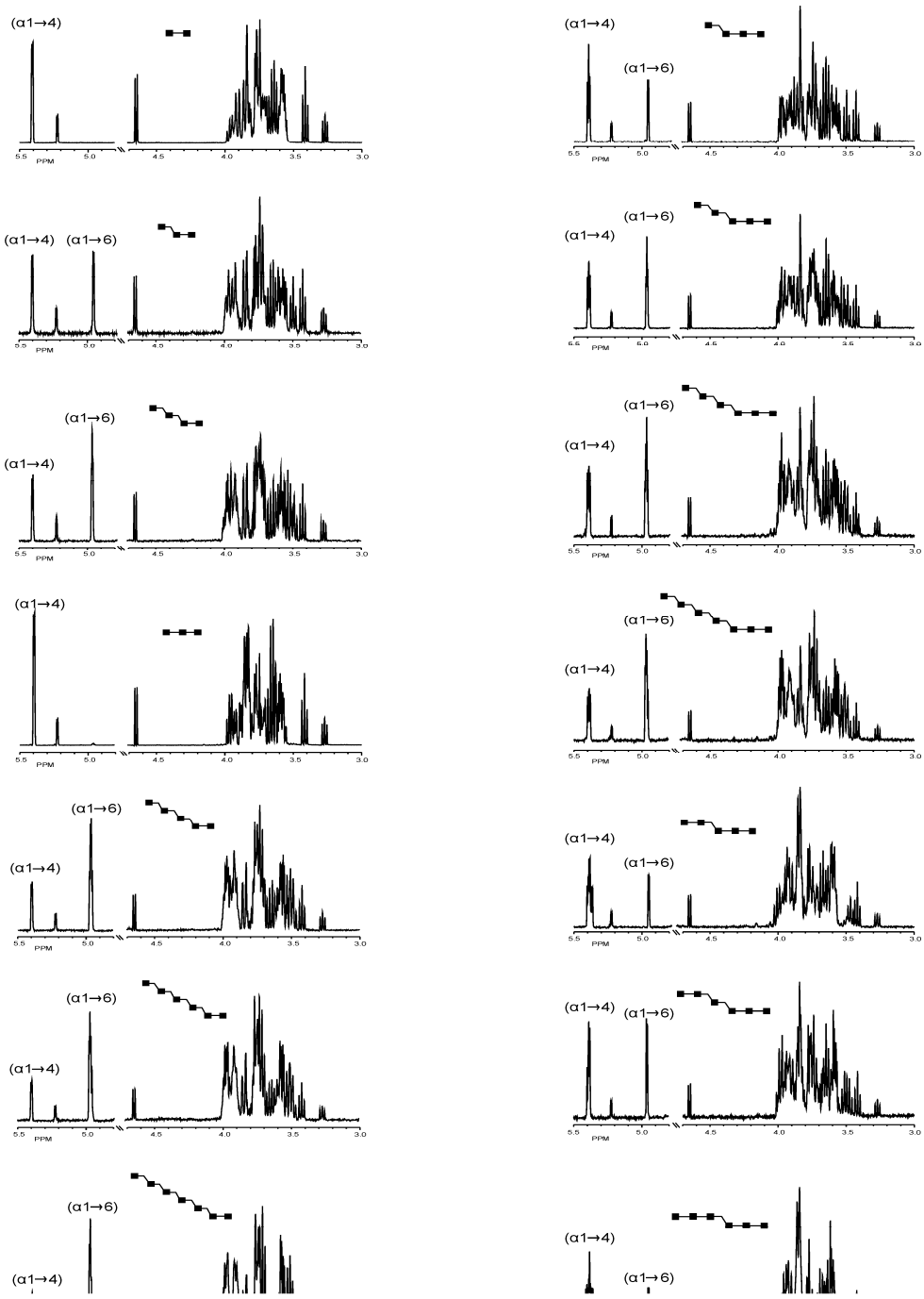

Fig. S2. ^{1}H NMR spectra of the products found in the generated oligosaccharide mixtures after the incubation of maltose and/or maltotriose with GTFB for 72 h at 37°C and pH 4.7. Note that the reducing Glc units occur as α/β mixture.

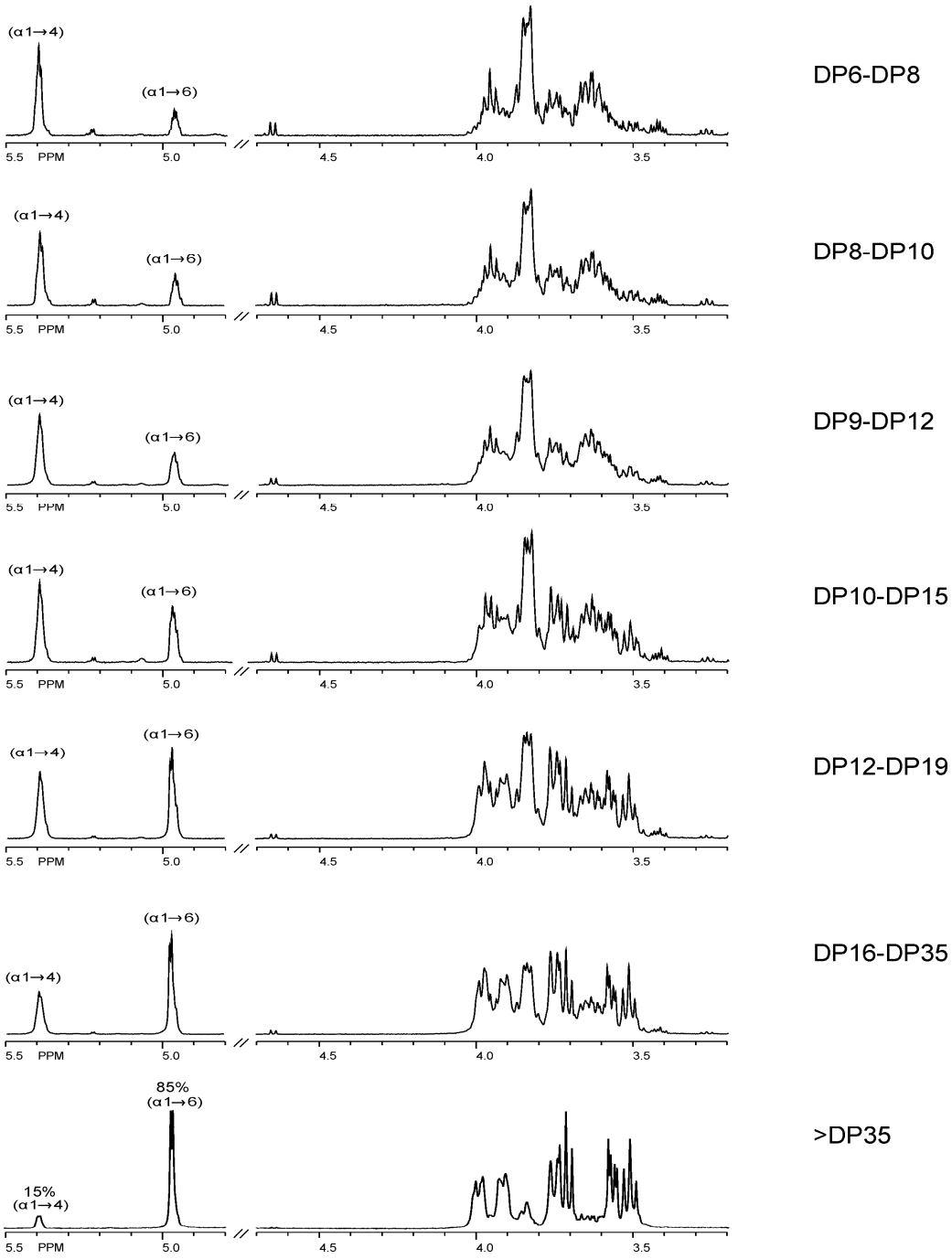

Fig. S3. One-dimensional ^{1}H NMR spectra of different Bio-Gel P-2 fractions with increasing DP (DP6–DP8, DP8–DP10, DP9–DP12, DP10–DP15, DP12–DP19, DP16–DP35, DP>35), obtained from the incubation of maltoheptaose (DP7) with GTFB, showing that the amount of (α1→6) linkages increases with increasing DP.

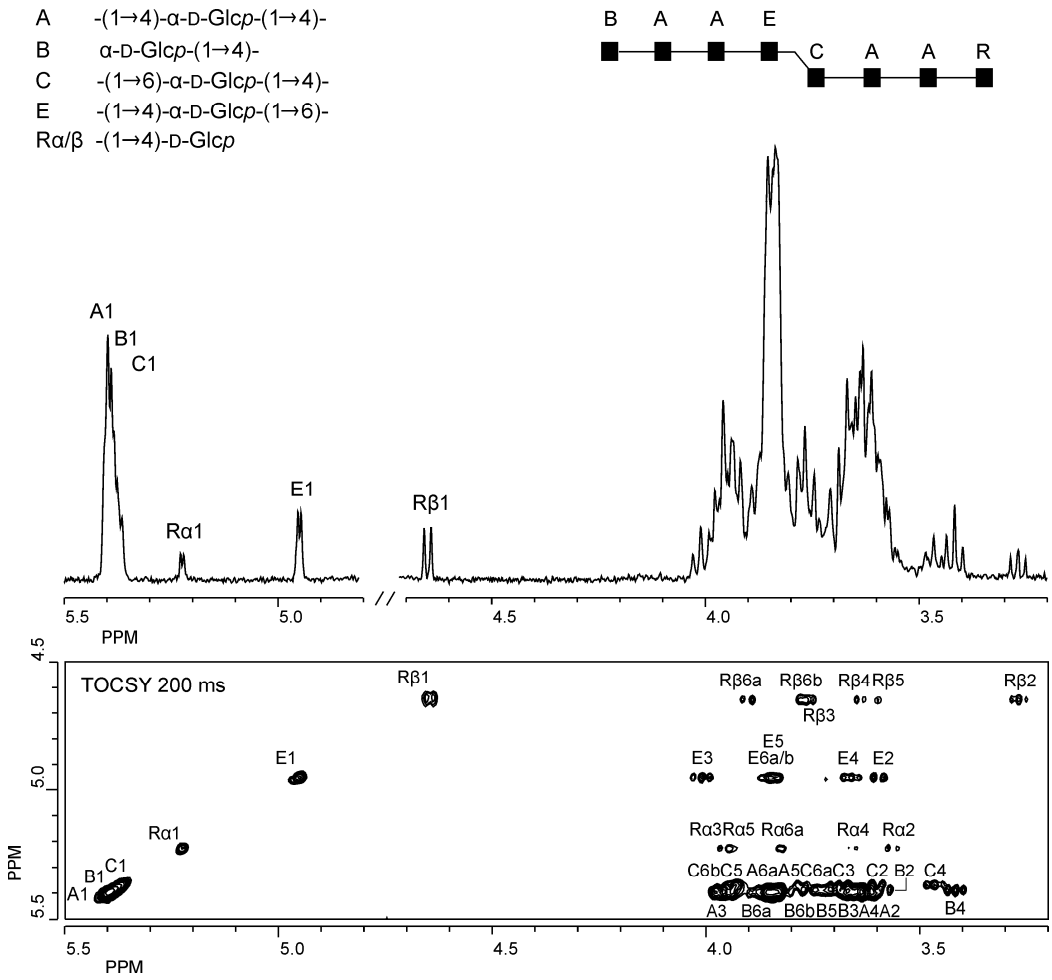

Fig. S4. One-dimensional ^{1}H NMR and TOCSY (200 ms) spectra of a DP8 product oligosaccharide [α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-D-Glc*p*].

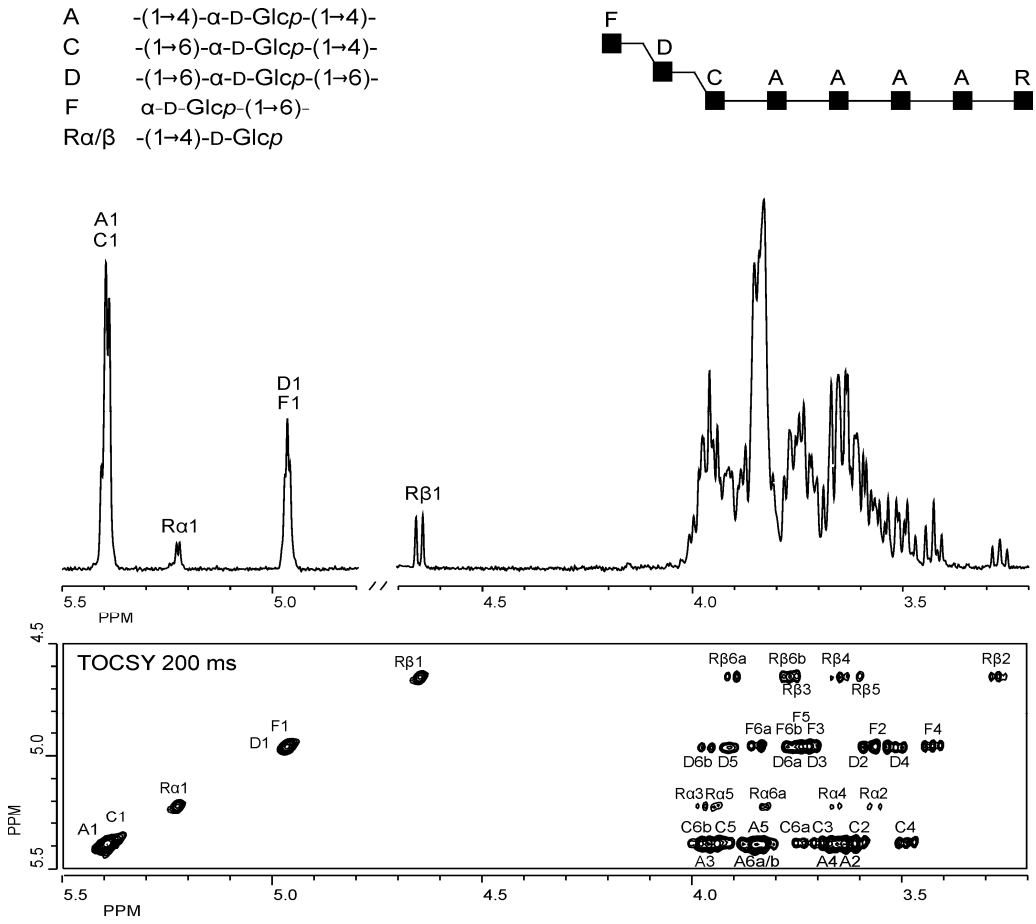

Fig. S5. One-dimensional ^{1}H NMR and TOCSY (200 ms) spectra of a DP8 product oligosaccharide [α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-D-Glc*p*]. Note the similarity of the TOCSY spectrum with those in Figure 5 and S6.

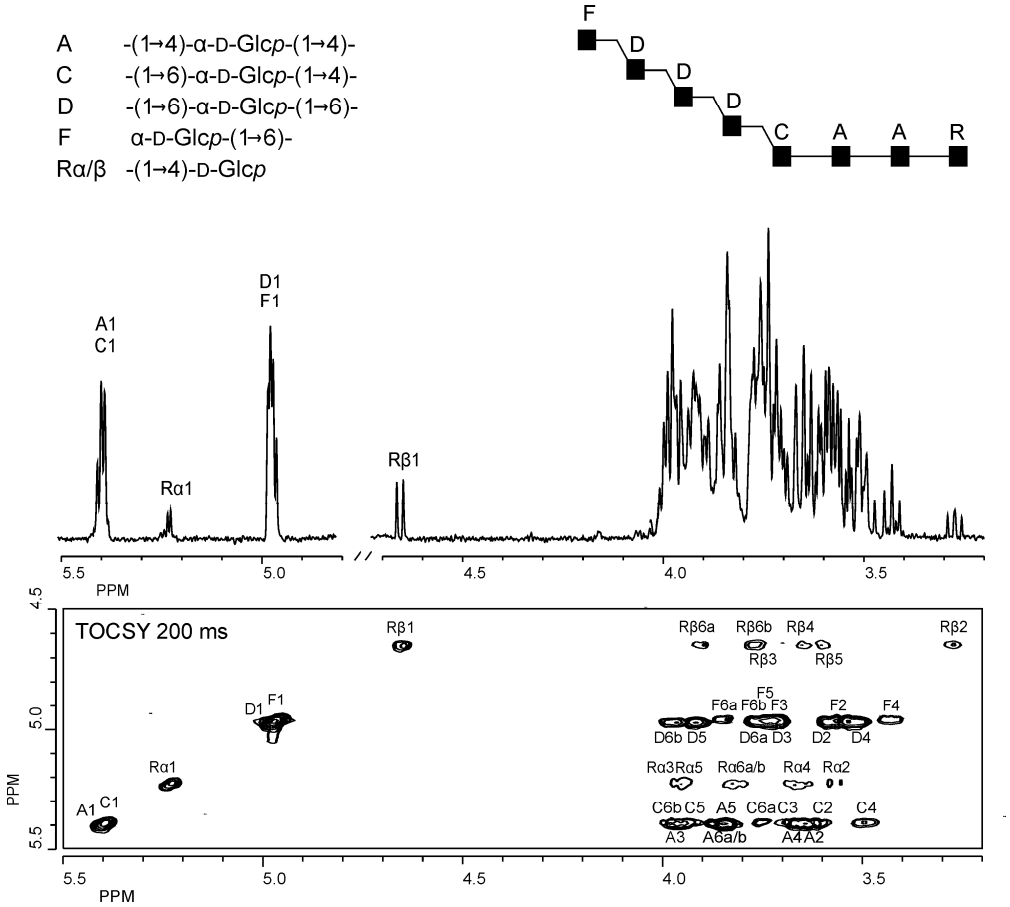

Fig. S6. One-dimensional ^{1}H NMR and TOCSY (200 ms) spectra of a DP8 product oligosaccharide [α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-D-Glc*p*].

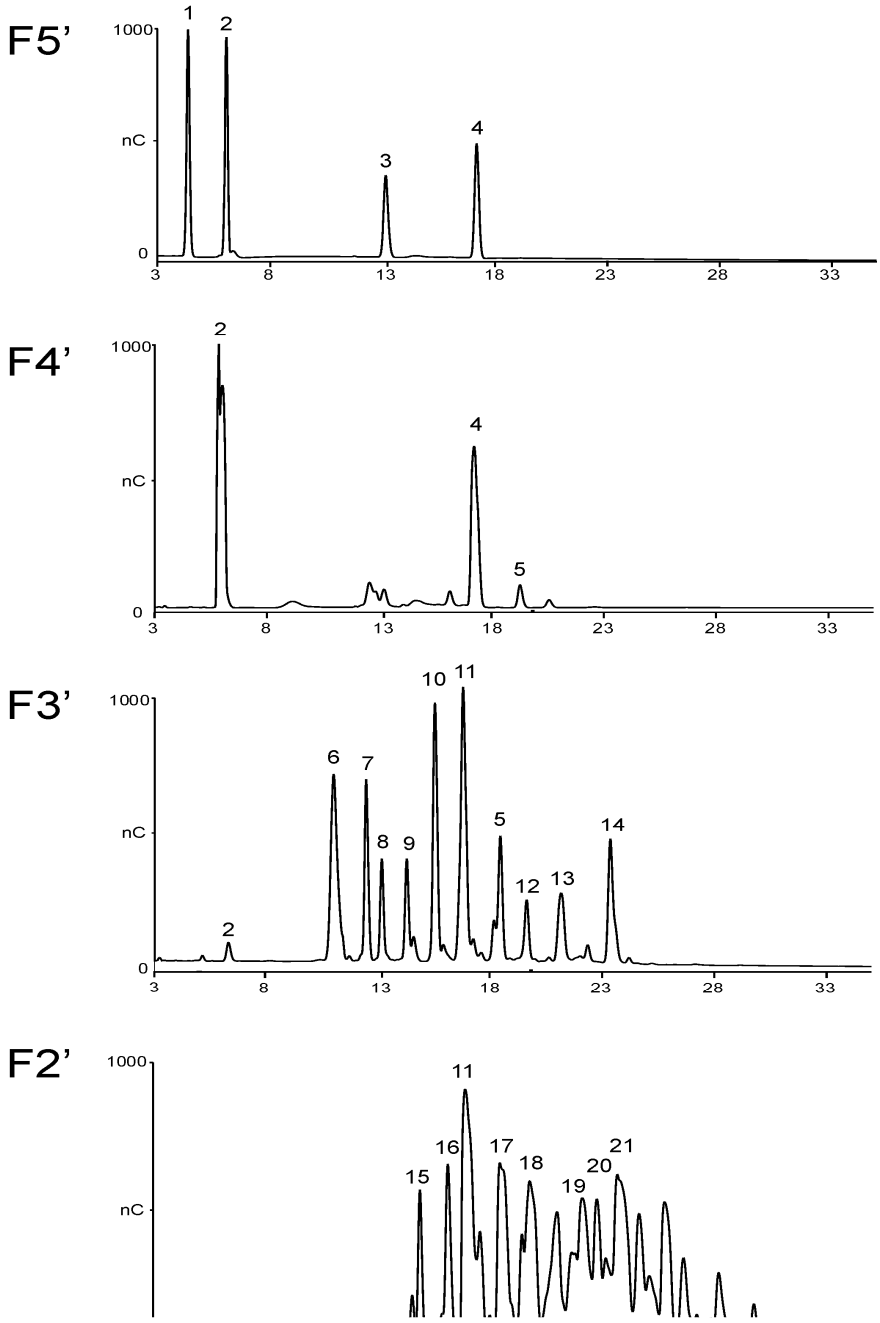

Fig. S7. HPAEC-PAD subfractionation (0 to 500 mM NaOAc gradient in 100 mM NaOH) of Bio-Gel P-2 fractions **F2′**-**F5′** on CarboPac PA-1 [incubation of maltopentaitol (DP5-ol) with GTFB].